REMARKS

Claims 14 and 30-33 are now pending in the application. Claims 14, 30, and 33 have been amended. No new matter is presented; as such the Amendment is proper under 37 C.F.R. § 1.116. Applicants respectfully request reconsideration of the claims as amended and passage to issuance.

I. Oath – Objections

Applicants acknowledge that the Oath of April 18, 2002, fails to claim priority to Provisional Application 60/267,447. Applicants respectfully request that this objection be held in abeyance pending allowance of the claims.

II. Claims – Objections

The Examiner objects to claim 30 for improper antecedent usage. Claim 30 has been amended on line 7 to change "a" to "the" and is now in proper form.

III. Claim Rejections – 35 U.S.C. § 112, Second Paragraph

The Examiner rejects claims 14 and 30-33 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The Examiner states that the limitation whereby the baseline fluorescence of a cell is measured prior to the introduction of the nucleic acid molecule encoding the fluorescent chimeric protease substrate is new matter. Claims 14 and 30 have been amended to omit this limitation. Claims 14 and 30 now include a limitation whereby the fluorescence of a cell expressing the chimeric protein is "compared to a control cell selected from the group consisting of: a control cell lacking active protease and a control cell expressing a green fluorescent protein reporter lacking said protease substrate."

Applicants submit that measuring change in fluorescence relative to a control cell lacking active protease or a control cell expressing a green fluorescent protein reporter lacking said protease substrate is supported by the specification and is not new matter. Comparison to a control cell lacking active protease is shown in Figure 2. Applicants teach expression of the chimeric protein in cells where the protease is active versus cells where the protease is inactive due to an amino acid substitution (NS3/4A S139G, Figure 2). The specification states that "[b]acteria expressing the mutant protease displayed fluorescence similar to GFP_N alone. However, the fluorescence of GFP_N in bacteria co-expressing NS3/4A was quenched completely" (specification, page 7). Comparison to a control cell expressing a green fluorescent reporter protein lacking active protease is also shown in Figure 3. Applicants teach expression of the chimeric protein in cells where the protease is not present because expression has not been induced (Figure 3, lane 3) compared to conditions where expression of the protease is induced (Figure 3, lane 4). Induction of the protease exhibited cleavage of the substrate compared to the uninduced control.

Comparison to a control cell expressing a green flouorescent reporter protein lacking said protease substrate is shown in Figure 3. Applicants teach expression of wild-type GFP (Figure 3, lane 1), which does not contain a protease substrate insert, is not cleaved by the protease, whereas cleavage of the GFP_N substrate by NS3/4A is observed. Moreover, Applicants teach expression of GFP_N in the presence of a NS3/4A S139G mutant (Figure 2). GFP_N does not contain the protease substrate for the NS3/4 S139G mutant. Consequently, the GFP_N polypeptide retrains fluroescence when in the presence of NS3/4A S139G (Figure 2).

The Examiner rejects claim 33 for being dependent on itself. Claim 33 has been amended to correct this error. Applicants thank the Examiner for pointing out this inadvertent mistake.

Applicants submit that the amendments to claims 14, and 30-33 alleviate the rejections under 35 U.S.C. § 112, second paragraph, and respectfully request reconsideration.

IV. Claim Rejections – 35 U.S.C. § 112, First Paragraph

The Examiner rejects claims 14 and 30-33 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner states that the limitation whereby the baseline fluorescence of a cell is measured prior to the introduction of the nucleic acid molecule encoding the fluorescent chimeric protease substrate is new matter. Claims 14 and 30 have been amended, as discussed in the preceding section, thereby alleviating this rejection. Applicants submit that the amendments to claims 14, and 30-33 alleviate the rejections under 35 U.S.C. § 112, first paragraph, and respectfully request reconsideration.

V. Claim Rejections – 35 U.S.C. § 103

The Examiner rejects claim 14 under 35 U.S.C. § 103(a) as unpatentable over Mahajan et al, in view of Abedi et al, and rejects claims 30-33 under 35 U.S.C. § 103(a) as being unpatentable over Mahajan in view of Abedi and further in view of Martin et al and Grakoui et al. Applicants respectfully traverse these rejections and request that the Examiner reconsider and withdraw the rejection based on the following additional arguments.

Applicants assert that that Examiner has not established a *prima facie* case of obviousness. The Examiner has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ 2d 1596, 1598 (Fed. Cir. 1988). In order for the Examiner to establish a *prima facie* case of obviousness, three base criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success.

Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *M.P.E.P.* § 2142 (citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed.Cir. 1991)).

The Examiner states in the Office Actions of September 23, 2003, and the March 10, 2004, that one would be motivated to use a fluorescent substrate comprised of only GFP, rather than CFP plus GFP for two reasons: (1) the GFP-peptide substrate would be smaller and therefore less prone to random proteolysis and (2) one would want to avoid bleed-through background fluorescence, which would occur upon cleavage of the CFP-peptide-GFP fluorescent substrate to generate free CFP and GFP.

The Applicants respectfully point out that these are not problems exhibited by the prior art references. First, there is no indication in Mahajan that the fluorescent substrate is more susceptible to random proteolysis because of its size. The constructs in the instant reference provide expression in both bacteria and mammalian cells that is high and clearly adequate for measuring a change in FRET signal caused by proteolysis (see Figure 2 and Figure 5). Second, the combined CFP-peptide-GFP substrate is approximately 55 kDa in size. Considering that the average human protein size is 50 kDa, one of ordinary skill in the art would not expect that a protein of only 55 kDa would be more susceptible to random proteolysis compared to the 27 kDa GFP alone. Third, the prior art teaches that "*Aequorea*-related fluorescent proteins tend to be protease-resistant and tend to survive as fluorescent moieties even after the linker moiety is cleaved" (Tsien et al., U.S. Pat. 5,981,200, column 21, line 19-30). Since the CFP and GFP are independently resistant to proteolysis, the prior art teaches away from the idea that the two

proteins, even if joined together, would be more susceptible to proteolysis. Thus, the CFP-peptide-GFP construct would not present a problem of susceptibility to random proteolysis that would motivate one of ordinary skill in the art to combine the teachings of Mahajan and Abedi to render the instant invention obvious.

The Examiner also states that another motivation to combine the Mahajan and Abedi references is that one would want to avoid bleed-through background fluorescence, which would occur upon cleavage of the CFP-peptide-GFP fluorescent substrate to generate free CFP and GFP. There is no indication in Mahajan that background fluorescence is a problem with assays using the combined CFP-peptide-GFP substrate. Background fluorescence is a problem with traditional FRET analysis where the fluorescent proteins are typically fused to separate proteins, and interaction between those proteins is evidenced by an increase in FRET signal from the acceptor protein. Because weak interactions might exhibit only a low FRET signal, background (or bleed-through) fluorescence can interfere with the detection of that signal.

In contrast to the traditional FRET analysis, the construct taught by Mahajan initially links the two fluorescent proteins via a peptide bond. Protease activity is evidenced by a decrease in FRET signal, relative to that exhibited by the uncleaved substrate (see Figure 2). Bleed-through or background fluorescence from free GFP and CFP would be negligible compared to the intense FRET signal exhibited by the uncleaved substrate.

Accordingly, the problems identified by the Examiner that would provide the suggestion or motivation to combine prior art teachings are not applicable to the Mahajan reference. Absent a suggestion or motivation to combine prior art teachings, the claimed invention is inventive and not obvious over Mahajan in view of Abedi, or Mahajan in view of Abedi in further view of

Martin and Grakoui. Applicants respectfully request withdrawal of the rejection for claims 14 and 30-33.

Moreover, there is nothing in the references cited by the Examiner that teaches that cleavage of a GFP polypeptide results in loss of fluorescence from said polypeptide. The Mahajan reference teaches that cleavage of a protease substrate between two fluorescent proteins causes a loss of FRET signal and the Abedi reference teaches that placement of an aptamer at several sites within the protein can decrease fluorescence activity, but neither teaches the effect of cleavage of the GFP polypeptide. While it may be known to one of ordinary skill in the art that protease cleavage might affect the activity of GFP, it is by no means certain. Cleavage at different sites might have no effect or varying effects on fluorescence activity. The present invention claims a GFP reporter protein "wherein the presence of a peptide bond between an amino terminal portion and a carboxyl terminal portion of said protease substrate sequence is essential to generate or maintain fluorescence of said chimeric protein." None of the prior art references cited by the examiner teach that protease cleavage of GFP results in a loss of fluorescence. As the prior art does not teach all of the claimed limitations, the claimed invention is inventive and not obvious. Accordingly, Applicants request that the rejections to claims 14 and 30-33 based on 35 U.S.C. § 103(a) be withdrawn.

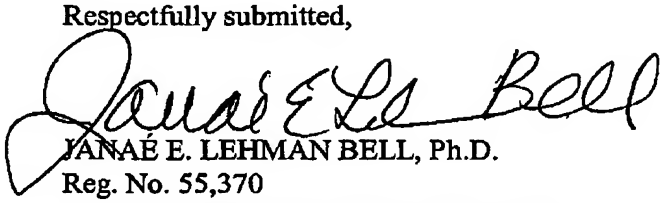
VI. Conclusion

Applicants submit that in light of the foregoing amendments and remarks, claims presented herein are in condition for allowance. Reconsideration is respectfully requested. If it is felt that it would aid in prosecution, the Examiner is invited to contact the undersigned at the number indicated to discuss any outstanding issues.

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,



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